ONLINE METHODS:

Fly stocks. Flies were reared at room temperature on conventional cornmeal agar. All experiments were performed on adult female flies 2-5 days post-eclosion. Fly stocks were kindly provided as follows: *Or43b*[1] (Dean Smith); *NP5103-Gal4, NP3481-Gal4*, and *NP5221-Gal4* (Kei Ito and Liqun Luo); *Or46a-Gal4* and *Or83b-Gal4* (Leslie Vosshall); *UAS-DTl*$_{II}$ and *UAS-DTl*$_{III}$ (Leslie Stevens); *UAS-PTX* (Gregg Roman); *UAS-CD8GFP*$_{I}$ (Bloomington Stock Center).

ORN recordings. Extracellular recordings of ORN spiking were performed as previously described[10]. In order to accurately sort spikes recorded from VC1 ORNs we killed the second ORN type (VA7l) housed in the same sensillum. This was done by expressing diphtheria toxin light chain in the VA7l ORNs (genotype: either *Or46a-Gal4/UAS-DTl*$_{II}$ (n = 4) or *Or46a-Gal4/+;UAS-DTl*$_{III}$/+ (n = 4)). The genotype for VM7 ORN recordings was *NP3481-Gal4,UASCD8GFP*, which is the same genotype we used for the VM7 PN recordings. VM7 ORNs fire spontaneously at 10 spikes/sec, and VC1 ORNs fire spontaneously at 3 spikes/sec.

PN recordings. *In vivo* whole-cell recordings from PNs were performed as previously described[8, 9]. For the current-clamp recordings in Figs. 1, 2, 5 and Supplementary Figs. 2-5, the composition of the internal patch-pipette solution was (in mM): potassium aspartate 140, HEPES 10, MgATP 4, Na_3GTP 0.5, EGTA 1, KCl 1, biocytin hydrazide 13 (pH = 7.3, osmolarity adjusted to ~ 265 mOsm). For the voltage-clamp recordings in Figs. 3-4 and Supplementary Figs. 6-8, the composition of the internal was (in mM): cesium aspartate 140, HEPES 10, MgATP 4, Na_3GTP 0.5, EGTA 1, KCl 1, biocytin hydrazide 13, QX-314 10 (pH = 7.3, osmolarity adjusted to ~ 265 mOsm). The composition of the external saline solution in all recordings was (in mM): NaCl 103, KCl 3, *N*-tris(hydroxymethyl) methyl-2-aminoethane-sulfonic acid 5, trehalose 8, glucose 10, $NaHCO_3$ 26, NaH_2PO_4 1, $CaCl_2$ 1.5, and $MgCl_2$ 4. Osmolarity was adjusted to 270-275 mOsm. The saline was bubbled with 95% O_2/5% CO_2 and reached a final pH = 7.3. Saline perfused the brain continuously at 2mL/min. Recordings were obtained with an A-M Systems Model 2400 amplifier (100 MΩ headstage), low-pass filtered at 5 kHz, and digitized at 10 kHz. Data was acquired in Igor Pro. In most experiments we used an enhancer trap line to label specific PNs with GFP for targeted recording. VM7 PNs are labeled by *NP3481-Gal4,UAS-CD8GFP* and VC1 PNs are labeled by *NP5221-Gal4,UAS-CD8GFP*. VM2 PNs are labeled by *NP5103-Gal4,UAS-CD8GFP*. VM2 PNs were recorded in flies in which the odorant receptor expressed by VM2 ORNs was mutated (*NP5103-Gal4,UAS-CD8GFP;Or43b*[1], see schematic in Fig. 3a). In some experiments for each genotype we filled the recorded cell with biocytin and verified the PN identity with biocytin histochemistry as previously described[8]. In the GABA iontophoresis experiments in Fig. 3d-f, we recorded from PNs in the anterodorsal cell cluster[5] labeled by the enhancer trap line *GH146* (*GH146-Gal4,UAS-CD8GFP*). For the experiments in which pertussis toxin was selectively expressed in ORNs (Fig. 4), we recorded from PNs in the anterodorsal cell cluster in the genotype *Or83b-Gal4;UAS-PTX/+*. In voltage clamp recordings, the command potential was either -85 mV (Fig. 3a-c) or -65 mV (Fig. 3d-f, Fig. 4).

Manipulation of peripheral organs. Antennal input was abolished in some experiments by severing the antennal nerves with fine forceps just prior to recording. The antennal nerve was gently broken by applying forces perpendicular to the long axis of the nerve, leaving a stump of nerve attached to the antennal lobe. In other experiments, the maxillary palps were removed with forceps just prior to recording. For both antennal and palp amputation, the cell bodies of the affected ORNs are removed, but the proximal portions of their axons remain intact and continue to innervate the antennal lobe. Amputation removes ORN somata and thus large spontaneous spike-driven EPSCs (~5-20 pA) disappear almost entirely. Axon terminals are still intact, as evidenced by the persistence of miniature EPSCs (~1 pA). In some experiments the maxillary palps were shielded from direct odor stimulation by covering them in “5 Minute Epoxy” (Devcon, Riviera Beach, FL). The epoxy was allowed to dry for ~20 min before beginning the recording. We confirmed that this substance

is relatively non-toxic to ORNs by covering the antennae in epoxy, allowing it to dry, peeling the epoxy away, and verifying that odors were still able to elicit a normal field-potential response in the antennae.

Olfactory stimulation. Odors were diluted in paraffin oil at a ratio of 1:100 v/v and were refreshed every 5 days. Our odor panel consisted of benzaldehyde, butyric acid, 1-butanol, cyclohexanone, ethyl butyrate , ethyl acetate, ethyl-3-hydroxybutryate, fenchone, geranyl acetate, 2-heptanone, linalool, 4-methyl cyclohexanol, methyl salicylate, 3-methylthio-1-propanol, 4-methyl phenol, octanal, 1-octen-3-ol, pentyl acetate, trans-2-hexenal, and paraffin oil (solvent control). Odor source details are posted at http://wilson.med.harvard.edu/odors.html. Odors were delivered with a custom-built olfactometer as previously described[10]. Odor stimuli were applied for 500 ms, except in Fig. 3 in which the odor was applied for 1s. For the experiments in Figs. 1, 2 and 5 consecutive odor presentations were spaced 25-35 s apart.

Direct ORN axon stimulation. In Figs. 3-4 and Supplementary Figs. 6-8 we electrically stimulated ORN axons. The ipsilateral antennal nerve was severed and inserted into a stimulating suction electrode. Single spikes were evoked in ORN axons with a short current pulse (50 μs) using a stimulus isolator (A.M.P.I., Jerusalem, Israel). In each sweep we delivered a train of 23 nerve stimuli at 4 Hz. During the train the size of the evoked EPSCs decreased over the first 4-8 stimuli due to short-term depression but then reached a steady value. We timed our GABA iontophoresis and odor stimulation to occur during this steady-state period. For the paired-pulse experiments in Supplementary Fig. 7 we delivered two electrical stimuli to the nerve with an inter-pulse interval of 25 ms. This interval produces depression at the ORN-PN synapse under control conditions and allowed us to observe increases in the paired-pulse ratio when we decreased release probability.

GABA iontophoresis. In the GABA iontophoresis experiments a high-resistance (~80 ΩM) sharp glass pipette was filled with a solution of 250 mM GABA in water, and the pH was adjusted to 4.3 by adding HCl. GABA was ejected into the antennal lobe neuropil with a brief (3-20 ms) positive current pulse using an iontophoresis unit (World Precision Instruments, Sarasota, FL). A constant negative backing current was applied in order to retain GABA in the iontophoresis pipette between ejection events. In order to ensure that a similar amount of GABA was released into the neuropil in all experiments, and that the time-course of GABA release was as consistent as possible, we adjusted the location of the iontophoresis pipette, the level of negative backing current, and the duration of the ejection current at the beginning of each experiment. We did not proceed with an experiment until the following criteria were met: 1) EPSC suppression was ~50% at 150 ms after iontophoresis, 2) maximal EPSC suppression was ~85% at 400 ms after iontophoresis, and 3) GABA evoked an outward current in the recorded PN which lasted for ~1-1.5 s.

Antagonists. Antagonists were prepared as concentrated stock solutions and a measured volume of stock was added to the saline perfusate to achieve the final drug concentrations. Picrotoxin (Sigma) was used at 5 μM, CGP54626 (Tocris) was used at 50 μM, $CdCl_2$ (Sigma) was used at 10-25 μM.

Data analysis.

Peri-stimulus time histograms. For the experiments in Figs. 1, 5 and Supplementary Figs. 2-3, spike times were extracted from raw ORN and PN recordings using routines in Igor Pro. Each cell was tested with multiple odors, and each odor was presented 6 times. The response to the first presentation was not included in our analysis. Each of the 5 remaining trials was converted into a peri-stimulus time histogram (PSTH) by counting the number of spikes in 50-ms bins that overlapped by 25 ms. These single-trial PSTHs were averaged together to generate a PSTH describing the response to an odor in a given experiment. Multiple cells corresponding to each glomerular class and each cell type (ORN or PN) were tested with a given odor in multiple experiments. Average PSTHs in Fig. 1 and Supplementary Figs. 2-3 represent the mean ± s.e.m computed across experiments.

Input-output functions. For the analysis of odor responses in Fig. 1d we computed the average spike rate over the 500-ms odor duration and subtracted the baseline firing rate averaged over the 500-ms window prior to odor onset. Each point in the input-output function represents the mean PN response to an odor plotted against the mean response of the cognate ORNs to the same odor. Fits are single exponential functions.

Lifetime sparseness. The selectivity of a neuron's odor response profile (Fig. 1e) was quantified as lifetime sparseness:

$$S = \frac{1}{1-1/N}\left(1 - \frac{\left(\sum_{j=1}^{N} r_j / N\right)^2}{\sum_{j=1}^{N} r_j^{\,2} / N}\right)$$

where N = the number of odors and r_j is the analog response intensity of the neuron to odor j minus baseline firing rate. Analog response intensity was the mean spike rate (averaged across 5 sweeps) during the entire 500-ms odor stimulus period. Any values of $r_j < 0$ were set to zero before computing lifetime sparseness (this was the only analysis in this study where negative responses were zeroed). Responses to paraffin oil (solvent control) were not considered in the sparseness analysis. We computed the lifetime sparseness for each individual cell in our study for which we tested at least 12 of the odors in our set. We used the Mann-Whitney *U*-test to assess the significance of sparseness differences.

Correlations between ranked odor preferences. To compute correlations between ORN and PN odor preferences, we computed Spearman's rank correlation coefficient (ρ) on the odor response vectors of these neurons. This is a nonparametric measure that quantifies how well any monotonic function could describe the relationship between ORN and PN responses. We chose this rather than a linear (Pearson's) correlation coefficient because the intrinsic input-output function of these glomeruli is highly nonlinear (Fig. 1d). For each glomerulus we computed Spearman's ρ on each pairwise combination of individually recorded ORNs and PNs for which we tested at least 12 of the same odors on both cells (glomerulus VM7: $n = 27$ comparisons for ORNs compared to PNs with antennae, $n = 38$ for ORNs compared to PNs without antennae; glomerulus VC1: $n = 19$ for ORNs compared to PNs with antennae, $n = 25$ for ORNs compared to PNs without antennae). We report the mean of these pairwise correlations. We assessed the significance of differences in Spearman's ρ with the Mann-Whitney *U*-test.

Quantifying changes in membrane potential. In Fig. 2c and Fig. 5c we show membrane potential responses averaged across multiple experiments. To generate these we first averaged 4-6 consecutive sweeps within a single experiment for each odor and low-pass filtered this at 13 Hz to remove any spikes. Next we averaged these responses across experiments, with ± s.e.m. shown in pastel. To quantify the magnitude of lateral input in Fig. 2d-f we integrated the change in membrane potential (versus baseline) over a 2-sec interval beginning at odor onset. We chose this interval because shorter intervals did not capture as much odor dependence in the magnitude of lateral input. In panels 2d and 2f, PN disinhibition was computed by averaging PN firing rates over the 2 sec following odor onset, then averaging across experiments (4-12 PNs per odor), before computing the difference in the PN responses with antennae versus without antennae. In panel 2e, total ORN activity for each odor was computed by summing all the response rates in ref.[23]. There are only 14 data points in this panel because only 14 of our odors were included in ref. [23].

EPSC amplitudes. We measured EPSC amplitudes in Figs. 3-4 and Supplementary Figs. 6-8 by first averaging together a block of 8-20 sweeps from the same experiment. From these averaged traces we computed the EPSC amplitude as the average over a 0.9-ms window centered on the peak. Before averaging across experiments, we normalized the train of evoked EPSCs to the baseline EPSCs evoked just prior to either odor or GABA onset.